

WHAT IS CLAIMED IS:

1. A method for isolating a polypeptide of interest comprising;
a) contacting a modified Fluorescein arsenical helix binder (FAsH) compound immobilized on a solid support with a solution containing a polypeptide of interest, which has been modified to contain a FAsH target sequence motif, under conditions that allow binding of the polypeptide to the immobilized FAsH compound; and
b) eluting the polypeptide of interest from the immobilized FAsH compound.
2. The method of claim 1, wherein the FAsH compound has been modified by acylation with an amino acid.
3. The method of claim 2, wherein the modification is by acylation with β -Alanine.
4. The method of claim 1, wherein the polypeptide of interest has been modified by the addition of the FAsH target sequence motif C C X₁ X₂ C C, where X₁ and X₂ are any amino acid.
5. The method of claim 4 wherein X₁ and X₂ are the same amino acid.
6. The method of claim 4 wherein X₁ and X₂ are different amino acids.
7. The method of claim 4 wherein the sequence motif has been added at either the N terminus or C terminus of the polypeptide, or in an alpha-helical region of the polypeptide.
8. The method of claim 1, wherein said solid support is selected from the group consisting of agarose, polyacrylimide, glass, ceramics, natural or synthetic polymeric materials, beads, coverslips, paper, metals, metalloids, polyacryloylmorpholide, various plastics and plastic copolymers such as NylonTM, TeflonTM, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon,

nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and controlled-pore glass, aerogels, and affinity exchange resins.

9. The method of claim 1, wherein the polypeptide of interest is eluted from the immobilized FIASH compound using a dithiol solution

10. The method of claim 9, where the dithiol solution is selected from the group consisting of 1,2 Ethanedithiol (EDT), Dithiotheritol (DTT), 2,3 and Dimercaptopropanesulfonate (DMPS).

11. The method of claim 1, wherein said solution which contains the polypeptide of interest is selected from the group consisting of cell lysate, crude polypeptide extract, and partially purified polypeptide extract.

12. The method of claim 11, wherein said solution is obtained from a cell or cell free solution derived from the group consisting of a plant, a prokaryote, and a eukaryote.

13. A DNA construct comprising an origin of replication, a selectable marker, a promoter that allows expression of the polypeptide of interest, and a multiple cloning site, wherein at the 5' end of the multiple cloning site is a genetically-encoded affinity tag, and wherein at the 3' end of the cloning site there is a FIASH target sequence motif.

14. The DNA construct of claim 13, wherein the selectable marker is selected from the group consisting of antibiotic resistance genes, fluorescent protein genes, and genes encoding protein toxins.

15. The DNA construct of claim 13, wherein the genetically encoded affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.

16. The DNA construct of claim 15, wherein the polyhistidine tag is the 6xhistidine (His6) tag.

17. The DNA construct of claim 13 wherein the FAsH target sequence motif has the amino acids C C X₁ X₂ C C.
18. The DNA construct of claim 17 wherein X₁ and X₂ are the same amino acid.
19. The DNA construct of claim 17 wherein X₁ and X₂ are different amino acids.
20. The DNA construct of claim 13, wherein the origin of replication, selectable marker, and promoter function in prokaryotic cells
21. The DNA construct of claim 13, wherein the origin of replication, selectable marker, and promoter function in eukaryotic cells.
22. The DNA construct of claim 13, wherein the origin of replication, selectable marker, and promoter function in insects cells.
23. The DNA construct of claim 13, wherein the origin of replication, selectable marker, and promoter function in plant cells.
24. A DNA construct comprising an origin of replication, a selectable marker, a promoter that allows expression of the polypeptide of interest, and a multiple cloning site, wherein at the 5' end of the multiple cloning site is a FAsH target sequence motif, and wherein at the 3' end of the cloning site there is a genetically-encoded affinity tag.
25. The DNA construct of claim 24, wherein the selectable marker is selected from the group consisting of antibiotic resistance genes, fluorescent protein genes, and genes encoding protein toxins.
26. The DNA construct of claim 24 wherein the FAsH target sequence motif has the amino acids C C X₁ X₂ C C.
27. The DNA construct of claim 26 wherein X₁ and X₂ are the same amino acid.

28. The DNA construct of claim 26 wherein X_1 and X_2 are different amino acids.
29. The DNA construct of claim 24, wherein the genetically encoded affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.
30. The DNA construct of claim 29, wherein the polyhistidine tag is the 6xhistidine (His6) tag.
31. The DNA construct of claim 24, wherein the origin of replication, selectable marker, and promoter function in prokaryotic cells
32. The DNA construct of claim 24, wherein the origin of replication, selectable marker, and promoter function in eukaryotic cells.
33. The DNA construct of claim 24, wherein the origin of replication, selectable marker, and promoter function in insects cells.
34. The DNA construct of claim 24, wherein the origin of replication, selectable marker, and promoter function in plant cells.
35. A method for producing a polypeptide of interest which has at its N-terminus a genetically-encoded affinity tag and at its C-terminus a FLASH target sequence motif comprising:
 - a) expressing a DNA sequence which encodes the polypeptide of interest from a DNA construct of claim 13 in an appropriate cell type; and
 - b) producing the polypeptide of interest from the cells of step a).
36. The method of claim 35, wherein the genetically encoded affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.
37. The method of claim 36, wherein the polyhistidine tag is the 6xhistidine (His6) tag.

38. A method for isolating a polypeptide of interest, which has at its N-terminus a genetically encoded affinity tag and at its C-terminus a FLAsH sequence motif comprising;

a) contacting a solution which contains a polypeptide of interest with an affinity resin which binds to the affinity tag;

b) eluting the polypeptides bound to the affinity column;

c) contacting the modified FLAsH compound immobilized on a solid support with the polypeptides from step b), under conditions that allow binding of the polypeptide to the FLAsH compound; and

d) eluting the polypeptide of interest from the immobilized FLAsH compound.

39. The method of claim 38, wherein the affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.

40. The method of claim 39, wherein the polyhistidine tag is the 6xhistidine (His6) tag.

41. The method of claim 38, wherein the FLAsH compound is immobilized to a solid support selected from the group consisting of agarose, polyacrylimide, glass, ceramics, natural or synthetic polymeric materials, beads, coverslips, paper, metals, metalloids, polacryloylmorpholide, various plastics and plastic copolymers such as NylonTM, TeflonTM, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and controlled-pore glass, aerogels, and affinity exchange resins.

42. The method of claim 38, wherein the polypeptide of interest is eluted from the affinity resin with a solution of imadizole.

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polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and controlled-pore glass, aerogels, and affinity exchange resins.

51. The method of claim 46 wherein the polypeptide of interest is eluted from the FLAsH compound using a dithiol solution.

52. The method of claim 51 wherein said dithiol solution is selected from the group consisting of 1,2 Ethanedithiol (EDT), Dithiotheritol (DTT), 2,3 and Dimercaptopropanesulfonate (DMPS).

53. The method of claim 46, wherein the affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.

54. The method of claim 53, wherein the polyhistidine tag is the 6xhistidine (His6) tag.

55. The method of claim 46, wherein the polypeptide of interest is eluted from the affinity resin with a solution of imadizole.

56. The method of claim 46, wherein the polypeptide of interest is eluted from the affinity resin with a low pH solution.

57. A kit comprising a modified FLAsH compound immobilized on a solid support.

58. The kit of claim 57, wherein the FLAsH compound has been modified by acylation with an amino acid.

59. The kit of claim 58, wherein the modification is by acylation with β -Alanine.

60. The kit of claim 57 wherein the modified FLAsH compound is immobilized on a solid support and pre-packaged for column chromatography.

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61. The kit of claim 60 where the column is prepared for a type of chromatography selected from the group consisting of batch chromatography, FPLC, HPLC, affinity chromatography and gel filtration.
62. The kit of claim 58 where the modified FIASH compound is immobilized on a solid support selected from the group consisting of agarose, polyacrylimide, glass, ceramics, natural or synthetic polymeric materials, beads, coverslips, paper, metals, metalloids, polacryloylmorpholide, various plastics and plastic copolymers such as NylonTM, TeflonTM, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and controlled-pore glass, aerogels, and affinity exchange resins.
63. The kit of claim 57, further comprising a dithiol solution for eluting the polypeptide of interest from the immobilized modified FIASH compound.
64. The kit of claim 63 wherein said dithiol solution is selected from the group consisting of 1,2 Ethanedithiol (EDT), Dithiotheritol (DTT), 2,3 and Dimercaptopropanesulfonate (DMPS).
65. The kit of claim 57 further comprising reagents and materials to prepare affinity resin columns.
66. The kit of claim 65 wherein the columns prepared are for a type of chromatography selected from the group consisting of batch chromatography, FPLC, HPLC, affinity chromatography and gel filtration.
67. The kit of claim 66 where the modified FIASH compound is immobilized on a solid support selected from the group consisting of agarose, polyacrylimide, glass, ceramics, natural or synthetic polymeric materials, beads, coverslips, paper, metals, metalloids, polacryloylmorpholide, various plastics and plastic copolymers such as NylonTM, TeflonTM, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF),

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silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and controlled-pore glass, aerogels, and affinity exchange resins.

68. The kit of claim 65 further comprising a solution or reagent for eluting the polypeptide of interest from the affinity resin.

69. The kit of claim 68 wherein said solution or reagent is an imadizole solution.

70. The kit of claim 68 wherein said solution or reagent is a low pH solution.

71. The kit of claim 65 further comprising a solution or reagent necessary for eluting the polypeptide of interest from the immobilized FAsH compound.

72. The kit of claim 71 wherein said solution is a dithiol solution.

73. The kit of claim 72 wherein said dithiol solution is selected from the group consisting of 1,2 Ethanedithiol (EDT), Dithiotheritol (DTT), 2,3 and Dimercaptopropanesulfonate (DMPS).

74. A method for producing a polypeptide of interest which has at its N-terminus a FAsH target sequence motif and at its C-terminus a genetically-encoded affinity tag comprising:

a) expressing a DNA sequence which encodes the polypeptide of interest from a DNA construct of claim 24 in an appropriate cell type; and

b) producing the polypeptide of interest from the cells of step a).

75. The method of claim 74, wherein the genetically encoded affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.

76. The method of claim 75, wherein the polyhistidine tag is the 6xhistidine (His6) tag.

77. A method for isolating a polypeptide of interest, which has at its N-terminus a FLaSH target sequence motif and at its C-terminus a genetically-encoded affinity tag comprising;

a) contacting a solution which contains a polypeptide of interest with an affinity resin which binds to the affinity tag;

b) eluting the polypeptides bound to the affinity column;

c) contacting the modified FLaSH compound immobilized on a solid support with the polypeptides from step b), under conditions that allow binding of the polypeptide to the FLaSH compound; and

d) eluting the polypeptide of interest from the immobilized FLaSH compound.

78. The method of claim 77, wherein the affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.

79. The method of claim 78, wherein the polyhistidine tag is the 6xhistidine (His6) tag.

80. The method of claim 77 wherein the polypeptide of interest has been modified by the addition of the FLaSH target sequence motif C C X₁ X₂ C C where X₁ and X₂ are any amino acid.

81. The method of claim 77 wherein X₁ and X₂ are the same amino acid.

82. The method of claim 77 wherein X₁ and X₂ are different amino acids.

83. The method of claim 77, wherein the FLaSH compound is immobilized to a solid support selected from the group consisting of agarose, polyacrylimide, glass, ceramics, natural or synthetic polymeric materials, beads, coverslips, paper, metals, metalloids, polacryloylmorpholide, various plastics and plastic copolymers such as NylonTM, TeflonTM, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon,

nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and controlled-pore glass, aerogels, and affinity exchange resins.

84. The method of claim 77, wherein the polypeptide of interest is eluted from the affinity resin with a solution of imadizole.

85. The method of claim 77, wherein the polypeptide of interest is eluted from the affinity resin with a low pH solution.

86. The method of claim 77 wherein the polypeptide of interest is eluted from the FIAsh compound using a dithiol solution.

87. The method of claim 86 wherein said dithiol solution is selected from the group consisting of 1,2 Ethanedithiol (EDT), Dithiotheritol (DTT), 2,3 and Dimercaptopropanesulfonate (DMPS).

88. A method for isolating a polypeptide of interest, which has at its N-terminus a FIAsh target sequence motif and at its C-terminus a genetically-encoded affinity tag comprising;

a) contacting the solution which contains a polypeptide of interest with a FLaSH compound immobilized to a solid support;

b) eluting the polypeptides bound to the immobilized FLASH compound;

c) contacting an affinity resin with the polypeptide solution from step b, under conditions that allow binding of the polypeptide to the affinity resin; and

d) eluting the polypeptide of interest from the affinity resin.

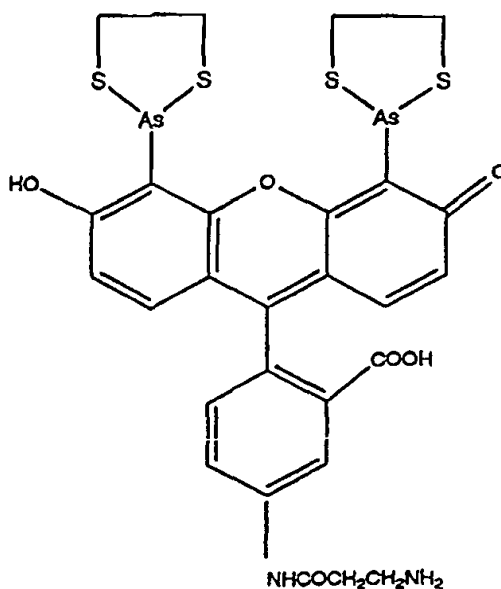
89. The method of claim 88 wherein the polypeptide of interest has been modified by the addition of the FLAsH target sequence motif C C X₁ X₂ C C where X₁ and X₂ are any amino acid.

90. The method of claim 88 wherein X₁ and X₂ are the same amino acid.

91. The method of claim 88 wherein X₁ and X₂ are different amino acids.

92. The method of claim 88 wherein the FLAsH compound is immobilized to a solid support selected from the group consisting of agarose, polyacrylimide, glass, ceramics, natural or synthetic polymeric materials, beads, coverslips, paper, metals, metalloids, polyacryloylmorpholide, various plastics and plastic copolymers such as NylonTM, TeflonTM, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polystyrene, polystyrene/latex, polymethacrylate, poly(ethylene terephthalate), rayon, nylon, poly(vinyl butyrate), polyvinylidene difluoride (PVDF), silicones, polyformaldehyde, cellulose, cellulose acetate, nitrocellulose, and controlled-pore glass, aerogels, and affinity exchange resins.
93. The method of claim 88 wherein the polypeptide of interest is eluted from the FLAsH compound using a dithiol solution.
94. The method of claim 93 wherein said dithiol solution is selected from the group consisting of 1,2 Ethanedithiol (EDT), Dithiotheritol (DTT), 2,3 and Dimercaptopropanesulfonate (DMPS).
95. The method of claim 88, wherein the affinity tag is selected from the group consisting of polyhistidine, maltose binding protein, glutathione S-transferase, and the FLAG tag.
96. The method of claim 95, wherein the polyhistidine tag is the 6xhistidine (His6) tag.
97. The method of claim 88, wherein the polypeptide of interest is eluted from the affinity resin with a solution of imadizole.
98. The method of claim 88, wherein the polypeptide of interest is eluted from the affinity resin with a low pH solution.

99. A modified fluorescein arsenical helix binding compound of the formula:



(I)

and tautomers, anhydrides, and salts thereof; wherein:

R is the product of an acylation reaction using any amino acid.

100. The compound of claim 99, wherein R is the product of an acylation reaction using β -alanine.

101. The compound of claim 99, wherein said compound specifically reacts with a target sequence when contacted with a polypeptide which contains a target sequence cable of being recognized by the compound of claim 99.

102. The compound of claim 99, wherein said molecule is substituted at one or more positions with a detectable group.

103. The compound of claim 99, wherein said compound is coupled to a solid phase.

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